BASIC GENETICAL AND IMMUNOLOGICAL CONSIDERATIONS

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The idea that means would one day be devised for transplantation of tissues and organs from one person to another, and perhaps even from lower animals to man, has long been part of the surgeon's creed. Nearly 400 years ago Ambroise Paré, in his *Apologie and Treatise*, included "to supply the defects of Nature" as one of the things he considered "proper to the duty of a chirurgian." And since the beginning of this century biologists from a wide variety of disciplines have been making increasing use of transplantation as a procedure for analyzing a wide range of problems. Indeed, during the past two decades increasing numbers of investigators have found that the elucidation of the requirements of grafts for survival and normal function, and contriving means of meeting these by experimental artifice, are worthwhile goals in themselves.

The purpose of this lecture is to present a brief outline of some of the important concepts and principles that underlie the multifaceted subject we have come to know as "transplantation." The central problems in transplantation are, of course, those that arise from the incompatibility of homografts (or allografts, as they are now properly called)—i.e., the innate and unrelenting intolerance of individuals to grafts of *other* people's tissues and organs. At the beginning of this century transplantation was being carried out mainly by two groups of individuals: (*i*) by plastic surgeons who, with a few neglected exceptions, confused themselves and others for many years by their failure to distinguish between *autografts* (i.e., grafts of which the *same* individual is both the donor and recipient, and which are permanently successful provided that certain purely technical requirements are satisfied) and *homografts*, and (*ii*) by cancer research workers, the majority of whom failed to recognize that the resistance which hosts manifested against grafts of tumor tissue was directed against them as foreign tissue *per se*, rather than against any specific properties associated with their malignant status.

Early studies on the fate of tumor grafts in mice soon established that the "race" of the host was an important parameter in determining whether they were accepted—i.e., survived—or otherwise, but the various findings were difficult to explain on a genetic basis. The problem was finally resolved by Dr. C. C. Little who, in 1914, postulated that acceptance of a tumor graft depends upon graft and host possessing a large number of genetically determined "susceptibility" factors in common. If the combination of susceptibility factors characterizing a particular tumor graft failed to match those of the host, the tumor would not grow properly.

Subsequent work by Little and Tyzzer and others soon placed this genetical theory of transplantation on a sound experimental footing, though neither Little nor his contemporaries expressed any views as to the mode of action of the susceptibility genes, or *histocompatibility genes*, as they are now known. Subsequent studies on mice by Dr. George D. Snell and his associates in the Jackson Laboratory at Bar Harbor gave precision and specific form to Little's genetic theory.

While the finer details of the genetics of tumor transplantation were being worked out at Bar Harbor, Dr. Peter Gorer, a London pathologist, was engaged in classic studies destined to help bridge the existing wide gap between knowledge of the genetic determination of tumor homograft incompatibility and the manner in which it was put into effect. In 1938, by serological procedures, he demonstrated the existence of antigenic differences between the red blood cells of mice of different genetically uniform or inbred strains, and that one antigen was common to the red cells, normal tissue cells and tumor cells of mice of one particular strain. When mice lacking this genetically determined antigen were confronted by tumor grafts bearing it, they produced antibodies directed against it. On the basis of this kind of evidence Gorer reformulated the genetic theory of transplantation in immunological terms as follows: "Normal and neoplastic tissues contain isoantigenic factors which are genetically determined. Isoantigenic factors present in the grafted tissues and absent in the host are capable of eliciting a response which results in destruction of the graft. Under special circumstances the response may not be elicited or the graft may not be destroyed thereby." Of course, as Gorer was aware, the antibodies he had discovered were not necessarily the agents responsible for graft destruction.

Subsequent work by Gorer and Snell and their respective associates, facilitated by the serologic approach, revealed that although there are many different genetically determined, histocompatibility systems in the mouse (upwards of 15), one of them, known as the H-2 locus, outweighs all the others in terms of its genetic complexity and the potency of the antigens it determines. It presents an astounding multiplicity of alleles, which themselves are composite, being made up of many closely linked pseudoalleles, with corresponding antigenic specificities. The most important property of this H-2 locus is its predominance over all the other histocompatibility loci with respect to the sensitizing or immunizing strength of its products—cellular transplantation antigens.

Subsequent investigations in other species, including chickens, rats, and man, have revealed that the histocompatibility systems of the mouse are indeed representative of histocompatibility systems in general. Each species seems to have its "major" locus, closely analogous to the H-2 locus, and many other minor loci as well. Of great clinical relevance is the fact that the intensity of a host's reaction against a homograft and the susceptibility of its reactivity to interference is predicated to a considerable extent by compatibility or otherwise at the major locus for the species concerned. The detailed knowledge of the mouse model and the availability of the serological techniques developed for its analysis have played an important role in the spectacularly rapid elucidation of the principal histocompatibility system in man—that which is determined by the HL-A locus—and the development of effective matching and typing procedures to be described in the following contribution by Dr. Bach.

Systematic study of homografts of normal, as opposed to malignant, tissues, dates back to Gibson and Medawar's careful comparison of the fates of skin homografts and autografts on a patient in 1943. This was followed by Medawar's classic work on skin homografts in rabbits, which established the basic pathophysiology of the homograft reaction and its immunological nature.

Justification for extending the conclusions drawn from studies on skin homografts to homografts of other tissues was provided in 1952 by the findings of independent studies of Dempster and Simonsen on the fate of renal homografts in the dog.

Progress in elucidating the details of the mechanism(s) that put transplantation immunity into effect has been exceedingly slow, and even now our knowledge of the "immediate" cause of death of both solid tissue and organ homografts is incomplete.

Awareness that infiltration of the substance of foreign grafts by leucocytes from the blood-stream—principally by lymphocytes and histiocytes—is a *constant* feature of the homograft reaction dates back to the beginning of the century. Indeed, a few students of tumor homografts did reach the conclusion that lymphoid cells are the principal agents of graft destruction. Unfortunately, they were unable to reconcile this "cellular" theory of homograft reactivity with the immunological thinking of their day, according to which all immunological processes or responses were supposed to be mediated by antibodies present in the serum. Early hopes that Gorer's isoantibodies would prove to be the effectors of homograft destruction in general were not sustained, although it was established that suspensions of cells of many types were susceptible to the complement-dependent action of these antibodies both *in vivo* and *in vitro*.

Passive transfer of heightened reactivity is one of the fundamental hallmarks of an immunologic response. However, it was not until 1954 that Mitchison established the important principle that immunity to homografts is transferable by means of regional lymph node tissue, but not by means of serum from mice that have rejected solid tumor homografts. This established that the regional nodes are the principal sites of a host's response against grafts that establish lymphatic connections with their hosts. It also opened up the "efferent" limb of the immunologic reflex to experimental study, and lent strength to a view that was slowly gaining ground: that homograft reactions, drug and bacterial allergies, and experimental autoimmune diseases are congeneric phenomena. They are all characterized by a dual immunologic response—cellular and humoral, and the cellular response is responsible for the lesions.

Following Mitchison's classic work on the transfer of transplantation immunity, it has been shown that cells capable of transferring transplantation immunity appear in the blood stream of animals that have received homografts. These cells have the morphological characteristics of small lymphocytes. Decisive evidence that transplantation immunity is a form of delayed hypersensitivity was presented by Brent, Brown, and Medawar in 1958. They demonstrated that, in the guinea pig, sensitivity evoked by skin homografts can express itself as a delayed cutaneous inflammatory reaction (the "Direct Reaction") closely resembling the classic tuberculin reaction, if a sensitized animal is injected intradermally with living donor cells or an antigenic extract prepared therefrom. This reactivity affords a useful means of measuring the intensity of homograft immunity and of assaying extracted putative transplantation antigens.

A new approach to the problem of the *modus operandi* of effector cells was opened up in 1960 by Govaerts, and by Rosenau and Moon, independently, using the simplified and definable milieu afforded by tissue culture. This work established the capacity of lymphoid cells from specifically sensitized animals to kill homologous "target" cells *in vitro* in the absence of added antibody or of complement. This work, in conjunction with the results of studies of local graft-versus-host reactions (see below), placed the postulated mediator role of "sensitized" lymphocytes on a sound experimental footing. It also highlighted another as yet unresolved question: By what means do infiltrating, sensitized lymphocytes procure the destruction of a foreign tissue or organ graft?

Localization and characterization of the cell constituents responsible for histoincompatibility is one of the most important facets of the subject. It is interesting to recall that on the basis of about 50 years' unsuccessful attempts to evoke resistance to homografts with non-living or disintegrated cells, the frustrating view was sometimes entertained that the capacity to elicit immunity to homografts was the prerogative of *living* cells. This unsatisfactory state of affairs was terminated and an element of "mystique" removed from transplantation immunology in 1956 when it was established that sensitivity against skin homografts in mice can be incited by pre-treatment with appropriately disintegrated spleen cells. Subsequently it was shown that the antigenic specificities determined by histocompatibility genes was associated with lipoprotein material of the plasma membranes of cells.

It is worth stressing that the greatest challenge in transplantation biology is to discover the significance of the fine-grained genetic polymorphism responsible for histoincompatibility. We are completely ignorant concerning the physiological or any other function of transplantation antigens. Is it fortuitous that these genetically variable molecules are antigenic if they are placed in a different host? Or does their immunologic individuality reflect their primary function?

The year 1950 opened a new era in transplantation in which the discovery of various means of weakening or abrogating the homograft reaction began to influence the direction of research in the field and engender increasing confidence that a solution to the problem of organ transplantation in man might not be too far off. It was in this year that Dempster, Lennox, and Boag reported the weakening influence of whole-body X-irradiation on the homograft reaction in rabbits, and in the following year it was established that the administration of cortisone prolonged the life-expectancy of skin homografts in this species.

The discovery of the principle of immunological tolerance—the state of specific immunological unresponsiveness of animals to homografts that follows their inoculation with donor cells very early in life (at or before birth)—came in 1953. This was a sequel to analyses of the immunogenetic consequences of synchorial vascular anastomoses, that occur naturally between multiple embryos in cattle, which had been initiated by Dr. Ray D. Owen in 1945. This early work on tolerance afforded a firm basis for belief that the induction of a state of *complete* specific nonreactivity of a person toward tissue or organ grafts from a particular donor, without necessarily prejudicing his capacity for immunologic defense, was a feasible objective. In the laboratory considerable progress has been made towards attainment of this goal. Complete tolerance of skin and other homografts has been achieved by the treatment of *adult* animals with living cells or antigenic extracts, facilitated in some cases by treatment with immunosuppressive agents.

Also in the early 1950's the important discovery was made that adult animals (usually mice) could be protected from potentially lethal doses of whole body X-irradiation by subsequent "grafts" of bone marrow from genetically similar or even dissimilar donors. Animals thus protected or, more correctly, rehabilitated become cell chimeras and will accept subsequent skin grafts from the donor strain which furnished the marrow cells.

Interest in the chemical approach to suppression of the homograft reaction was awakened by Dameshek and Schwartz's discovery that treatment of adult rabbits with the drug 6-mercaptopurine (6-MP) blocked their capacity to make antibodies against a foreign protein antigen, human serum albumin, and also impaired their capacity to reject skin homografts. Within two years the principle of chemical immunosuppression was successfully applied to renal homografts in dogs, and shortly thereafter to similar organs in man, as we shall hear from Dr. John Merrill.

It is paradoxical that what has proved to be the most potent and least harmful of all immunosuppressive agents in the laboratory—heterologous antilymphocyte serum, or ALS—has been under episodic investigation for about 70 years. ALS is an antiserum produced by injecting lymphocytes from a donor of one species into a recipient of a different species—e.g. mouse lymphocytes into rabbits. Injection of this agent into animals of the species against which the immunity is directed greatly impairs their capacity to react against homografts, without adversely affecting their capacity to protect themselves against infection by many different pathogenic microorganisms. Apart from its influence on the homograft reaction which was discovered by Woodruff and Anderson in 1963, ALS is very effective in prolonging the lives of *heterografts*, i.e., grafts of which donor and host belong to *different* species, with certain donor/host combinations. For example, it will enable human skin to flourish on mice for many weeks. It is conceivable that with the aid of ALS or, more likely, some greatly improved chemical immunosuppressant, it may in the future be possible to utilize organ grafts from domestic animals for therapeutic purposes in man, and so to ease the problem of donor organ procurement.

The discovery of graft-versus-host (GVH) reactions in 1957 opened a completely new vista in transplantation immunology. These reactions develop when living lymphoid cells, including cells that can react to antigens, are injected into hosts that confront them with foreign transplantation antigens and that, for some reason or another, are incapable of rejecting them. Under these conditions the cellular "graft" may proceed to react against its host from *within*, producing a severe and often fatal wasting disease, known as homologous, transplantation, or runt disease. Knowledge of graft-versus-host reactivity provided a timely warning of the possible hazards of transfusing immunologically competent cells into unmatched and immunosuppressed or otherwise immunologically debilitated human subjects.

Apart from systemic forms of graft-versus-host reactivity, *local* graft-versus-host reactions may develop when suspension of lymphocytes are inoculated into the skin or beneath the renal capsules of genetically appropriate hosts. Studies on these reactions have made several important contributions to our understanding of transplantation immunology. Most important of these is the fact that cells capable of initiating a host's response against a homograft are normal ingredients of its blood—small lymphocytes. This finding led to the suggestion that when a host's blood flows through the vasculature of a tissue or organ homograft these cells are "triggered-off" or activated to initiate the immune response.

It seems appropriate to conclude this introductory survey of transplantation biology by drawing attention to the fact that homotransplantation is not the sole prerogative of surgeons and experimental biologists. In all outbred populations of mammals, including man, embryos are homografts in the sense that they inherit transplantation antigens from their fathers against which their mothers are potentially capable of reacting. Nature's successful solution to her homograft problem, an essential prerequisite for the evolution on the mammals, has been shown to turn upon: (*i*) complete separation of the maternal and fetal blood circulations, and (*ii*) the presence of a complete and unbroken frontier layer of fetal cells in the placenta—the trophoblast—which, unlike nearly all other cells in the body, do not express their genetic endowment of transplantation antigens in an effective manner.